REMARKS

I. Support for the Amendments

Claims 1-26 were originally in the application. Non-elected claims 8-26 have been withdrawn. Claims 5 and 7 were canceled during previous Amendments.

Claims 1-4 and 6 were in the application. Claim 4 has been canceled without prejudice to its pursuit in an appropriate divisional or continuation application. Claims 1 and 3 have been amended. No new matter has been added by virtue of these amendments. Claims 1-3 and 6, as amended, are presently in the application.

Support for amended claims 1 and 3 can be found in the original specification, figures, and claims. The amendments to claims 1 and 3 have provided the sequence identifiers in accordance with the Examiner's request. Additional support for amended claims 1 and 3 can be found, e.g., from page 10, line 12, to page 11, line 5; from page 17, line 24, to page 18, line 8; in Figures 6 and 7; and in the Examples.

Support for the amendments to the specification can be found in the original specification, figures, and claims. The specification has been amended to provide sequence identifiers in accordance with the Examiner's remarks in the Office Action and to provide sequence identifiers for sequences previously provided in the Figures (particularly in Figures 5-7). Additional support for the amendment to page 26 of the specification can be found from page 22, line 17, to page 23, line 14, of the Japanese text of PCT/JP99/02305, a copy of which is provided herewith, with the relevant portions of the text underlined and indicated by check marks in the left-hand margin.

II. Status of the Claims

Claims 1-26 were originally in the application. Claims 1-26 were subject to an election/restriction requirement, and claims 1-7 were elected. Claims 8-26 were withdrawn without prejudice or disclaimer of any subject matter. Claims 4, 5, and 7 have been canceled. Claims 1-3 and 6 are presently in the application.

III. Nucleotide and/or Amino Acid Sequence Disclosures

The Examiner has required amendments to the claims with respect to the sequence identifiers and has requested a revised Sequence Listing, including both a paper copy and an electronic copy.

Applicants have amended claims 1 and 3 accordingly, in addition to the appropriate portions of the specification, in response to the Examiner's remarks and to provide sequence identifiers for sequences previously provided in the Figures (particularly in Figures 5-7). Applicants have requested the Examiner to enter the revised sequence listing.

Applicants respectfully submit that the amendments to claims, specification and sequence listing place the application in condition for allowance.

IV. Rejection of Claims 1-4 and 6 under 35 U.S.C. §112, First Paragraph

The Examiner has rejected claims 1-4 and 6 under 35 U.S.C. §112, first paragraph, for reasons relating to enablement. The rejection is rendered moot with respect to claim 4, which

has been canceled without prejudiced. Applicants respectfully traverse the rejection.

As amended, claim 1 recites a polypeptide having more than 50% identity with SEQ ID NO: 1 and comprising 101 conserved amino acid residues. These amino acid residues are commonly conserved in all amino acid sequences shown in Figure 7. Therefore, one of ordinary skill in the art would recognize the potential importance of these conserved amino acids, relative to the variable amino acids, with respect to the claimed nicotianamine synthase. As a result, use of part or all of the consensus sequence(s) in the present invention would not require undue experimentation on the part of one of ordinary skill in the pertinent art.

Applicants respectfully disagree for the reasons outlined *supra*, but have amended claim 1 in the interests of furthering the prosecution of the case. Support for the amendment to claim 1 can be found in Figure 7, as filed, and elsewhere. Claims 2, 3 and 6 are dependent on claim 1, and the reasoning that applies to claim 1 also applies to these claims. (Claim 4 has been canceled, rendering the rejection moot.) Applicants respectfully submit that the amendments place claims 1-3 and 6 in condition for allowance.

V. Correction of Inadvertent Error in the English Translation of the Specification

It has come to Applicants' attention that an inadvertent error occurred during the translation of the specification of PCT/JP99/02305 from Japanese into English. Applicants have amended the specification on page 26 of the English translation in order to correct the error.

First, in the translation filed with the application, the subject matter of original Example 9 was omitted, and original Example was incorrectly numbered and entitled as Example 9. Applicants have amended page 26 of the English translation to include the inadvertently omitted

text and to correct the titles of the Examples. Support for this amendment can be found from page 22, line 17, to page 23, line 14, of the Japanese text of PCT/JP99/02305. Copies of these pages are submitted herewith. The paragraph and title in question are underlined and indicated by check marks in the left-hand margin.

Second, in the text for Example 10 (as amended), the cited example number was inadvertently altered to "example 1" during the translation (see page 26, line 20). Applicants have amended page 26 to correct the cited example number to "example 9." Support for this amendment can be found on page 23, line 8, of the Japanese text of PCT/JP99/02305. A copy of this page is submitted herewith. The line is question is underlined and indicated by a check mark in the left-hand margin.

Applicants submit that these changes are supported in the original Japanese text of the international application (PCT/JP99/02305). The present case is a U.S. National Phase Application of PCT/JP99/02305 under 35 U.S.C. §371. Applicants respectfully submit that the amendment to the specification merely corrects an inadvertent error in translation, that the error was unintentional, and that no new matter is introduced into the application as a result of the amendment to correct the translation.

Applicants respectfully request the Examiner to enter the amendment correcting the English translation, which will place the application in condition for allowance.

CONCLUSION

In view of the foregoing amendments and remarks, the present application is respectfully considered in condition for allowance. An early reconsideration and notice of allowance are earnestly solicited.

It is believed that all outstanding rejections have been addressed by this submission and that all the claims are in condition for allowance. If discussion of any amendment or remark made herein would advance this important case to allowance, the Examiner is invited to call the undersigned as soon as convenient.

It is believed that a one-month extension of time is required. If a petition for an additional extension of time is required, then the Examiner is requested to treat this as a conditional petition for an additional extension of time. Although it is not believed that any additional fee, beyond the fee submitted herewith, is required to consider this submission, the Commissioner is hereby authorized to charge our deposit account no. 04-1105 should any fee be deemed necessary.

Respectfully submitted,

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ム社）に転写した。膜を０．５Ｍ　チャーチリン酸（Church and Gilbert 1984）１ｍＭ　ＥＤＴＡ、７％（ｗ／ｖ）ＳＤＳ、１００μｇ／ｍｌサケ精巣ＤＮＡを含むバッファーを用いて６５℃１晩でプローブとハイブリダイズした。これを４０ｍＭチャーチリン酸、１％（ｗ／ｖ）ＳＤＳを含むバッファーを用いて６５℃１０分間洗浄した。この洗浄をもう１回行った後、０．２ｘＳＳＰＥ、０．１％（ｗ／ｖ）ＳＤＳを含むバッファーを用いて６５℃１０分間洗浄した。放射活性はイメージアナライザーＢＡＳ－２０００で検出した。

結果を第９図及び第１６図に示す。

実施例８（サザンハイブリダイゼーション）

オオムギとイネの葉からそれぞれゲノムＤＮＡを抽出した。これをＢａｍＨＩ、あるいはＥｃｏＲＩ、あるいはＨｉｎｄＩＩＩで断片化し、０．８％（ｗ／ｖ）アガロースゲル電気泳動で分離した後、ハイボンドーＮ⁺膜（アマシャム社）に転写した。実施例７で述べた方法でハイブリダイズし、放射活性を検出した。

結果を第１０図に示す。

実施例９（ポリクローナル抗体の調製）

ネズミ２匹を約１００μｇの単離したニコチアナミン合成酵素を抗原として免疫した。抗原としては部分アミノ酸配列を決定したものと同じ試料を用いた。１回目の免疫時には完全フロイントアジュバント、２回目以降は不完全フロイントアジュバントを用いた。４回免疫した後、全採血を行い、血清を－８０℃で保存した。

実施例１０（ウエスタンブロット解析）

トリクロロ酢酸とアセトンを用いて全タンパク質を抽出した（Damerval et al. 1986）。植物体を液体窒素中で粉状になるまで粉砕し、１０％（ｗ／ｖ）トリクロロ酢酸、０．１％（ｖ／ｖ）２－メルカプトエタノール（2-mercaptoethanol）を含むアセトンと混合した。－２０℃で１時間静置してタンパク質を沈殿させた後、１６，０００ｘｇ　３０分遠心して沈殿を回収した。沈殿を０．１％（ｖ／

ｖ）２－メルカプトエタノール（2-mercaptoethanol）を含むアセトンに懸濁し、－２０℃で１時間静置してタンパク質を沈殿させた後、１６，０００ｘｇで３０分遠心して沈殿を回収した。沈殿を減圧乾燥した後、試料バッファー（９．５Ｍ尿素（urea），２％（ｗ／ｖ）トリトン－Ｘ－１００（Triton X-100），５％（ｖ／ｖ）２－ＭＥ）に溶かし、１６，０００ｘｇ１０分遠心して上清を得た。これに含まれるタンパク質をＳＤＳ－ＰＡＧＥ、あるいは変性２次元電気泳動（O'Farrell 1975）で分離した後、ＰＶＤＦ膜に転写した。この膜に対して、実施例９で調製した抗ニコチアナミン合成酵素抗体を１次抗体、西洋ワサビペルオキシダーゼ結合抗マウスＩｇＧ（Ｈ＋Ｌ）ヤギ抗体（和光純薬）を２次抗体として、ジアミノベンジジンで発色しウエスタンブロット解析を行った。

結果を第１２図に示す。ＳＤＳ－ＰＡＧＥは１２．５％アクリルアミドスラブゲルで行った。１００μｇのタンパク質を泳動した。根については２００μｇ、葉については５００μｇのタンパク質を泳動した。

実施例11（RT-PCR）

シロイヌナズナから全RNAを抽出し、その1 μｇを鋳型としてEZ rTth RNA PCRキット（パーキンエルマー社）を用いてRT-PCRを行った。プライマーはAtNAS1、AtNAS２、AtNAS３それぞれに特異的なものを用いた。結果を第１８図に示す。

産業上の利用可能性

本発明の組換えベクターを用いて種々の細胞を常法に従って形質転換することができ、得られた形質転換体を用いてニコチアナミドを大量に製造することができる。これらの方法は当業者に知られている方法により行うことができる。

また、本発明の遺伝子を用いて、植物、好ましくはイネ科植物の品種の改良を行うこともできる。特に、鉄分が欠乏している土壌においても生育できる品種に改良するために、本発明の遺伝子を利用することができる。